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THE RESISTANCE OF INERCTS TO ULTRA-LON TENGERATURES

by L. K. Lozins-Lozinskiy

- USSR -



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THE RESISTANCE OF INSECTS TO ULTRA-LOW TEMPERATURES

- USBR -

Following is the translation of an article by L. K. Lozina-Lozinskiy in the Russian-language publication Doklady Akademii Nauk SSSR (Reports of the Academy of Sciences USSR), Vol CXLVII, No 5, Moscow, 1962, pages 1247-1249.

(Presented by Academician Ye. N. Pavlovskiy on 3 May 1962.)

It has been established through our studies (1) that several species of insects can withstand freezing at the very low temperatures -55° and -78°. The pupae of Saturnia pyri consume oxygen for an extended period, but several eggs of the Asiatic locust Locusta migratoria preserved their normal appearance for many days following exposure of the eggs to a temperature of -183°. However, not all the caterpillars of the corn borer (Pyrausta nubilalis) following cooling down to -78° proved to be alive, and those which preserved their mobility gradually perished. The existence of respiration following chilling is not necessarily indicated by the fact that several signs of life were maintained in the organism. Only recently (2, 3) was it shown for several species of insects and other invertebrates that a certain percentage of the animals show motor reactions following chilling in liquid oxygen or nitrogen. The condition for survival at such temperatures is preliminary cooling at -30°.

Our investigations of 1961-1962, mainly on corn borer caterpillars have shown that preliminary cooling at -30° is not the necessary and only condition for survival at ultra-low temperatures. The highest cold-resistance is shown only by dispaused caterpillars (beginning from November). However, they also cannot withstand chilling down to -183° or -196°, if they are frozen during the initial period of the dispause or at its conclusion, when the period of reactivation has not yet set in. In these cases the preliminary cooling down to -30° does not promote survival.

In our experiments we succeeded in preserving full viability of the corn borer caterpillars following chilling at -78° and obtained a high percentage of live specimens following chilling

TABLE 1
Resistance of Caterpillars to -1960:
at -300 for 60 min; at -790 for 1 hr,
at -1960 for 1 day.

Me ceuton (B)	Natia ostuta 🕙	Vacao rycenny	Temeparyin care-	Post of the post o	жения к	Ilpanaranaects anse - Cyrax
(i)0	слаждая	H e: -		ije, - Tark	-79° 1 4	ac, —196°
19 20 27 29 27 52 53	15 X1 15 X1 15 X1 11 X11 11 X11 12 1 18 1 18 1	222222	300850640	0055 80000 A	C C 5 26 10 0 0 0	7 - 33 1 - 10 1 - 84 3 - CO G
(i) 2333333333	10 I 10 I 10 I 10 I 10 I 10 I 10 I 10 I	**************************************	35° 1	0 10 60 70 6.5 27 .8	0 10 5 5 0 18,7	0 3-4 3-26(10%) 5-26 4-26(14%) 0-4 4-20(16%) 4-20(16%)

LEGEND: a) No of experiment; b) Date of experiment; c) Number of caterpillars; d) Captivity temperature in oC; e) Reaction following thawing; f) number of active animals in %; number of animals reacting to elecshock in %°; h) Lifespan in days; i) Freezing; j) hour; k) day * Immobile caterpillars.

down to -196° under the following conditions. Diapaused corn borer caterpillars, caught in the Stavropol'skiy Kray, were placed under various temperature conditions for long periods (Table 1). Upon keeping the caterpillars at 0° and 4° for 3-4 weeks, their resistance to deep chilling rose sharply, the number of active caterpillars chilled first to -30° and then to -196°, following thawing was 60-70%, and those chilled down to -78° -- 100%. Caterpillars not previously cooled down to -30° survived at -78°, but perished at -183° and -196°. Without preliminary cold hardening at a temperature of around 0°, only a small percentage of the caterpillars survived following exposure to -78° and only individual caterpillars reacted to the electric stimulus after undergoing exposure to -196°. Temperatures below 0° do not promote hardening. The caterpillars, following preliminary deep chilling, were kept at a temperature of 22°, which promoted reactivation. However, as further experiments showed, the nighest survival rate of the caterpillars chilled down to -78° was reached when they were kept for a month at 0° (Table 2). In two

experiments, ll out of 30 chilled caterpillars chrysallized, resulting in eight butterflies -- males and females, some of which deposited eggs. Thus far, following such chilling only 50% proved to be active caterpillars, which after the period of one to one and a half months perished.

TABLE 2 Effect of Captivity Conditions Before and After Freezing on Caterpillar Resistance to -78°

€.	6	(C)	Температура со- держания в ° С		⑤	0	
MA CHATT	Aeru oeu	Tucas rye	Manual M	тое эконо замержая	Реакция после оттанвания	Выживаемость и развитие	
23	30 XI	10	22	22	5% реагируют на ал. тон()	Погибан черев і сут.	
22	30 X!	20	16	22	25% gentauren, 18% peerse pyiot na 84. tok	Ж или от 8 до 84 сут.	
24	30 X1	21	4	22	76.2% gaurantes, 14,8% pea- rappor sa as. Tou	Жиан от в до 65 сут.	
かご	56 X1	20	1 6	26 M XII 0, c 26 XII 23	100% двигаются	40% ONYMERACES, 30% 6250	
255	1X %	10	3	To me	160% двигаются	20% окуклилось, 20% бабо чек	
2 12	36 X1	10	-2	23	100% genratoren	Mush of 3 ge 25 evr.	

TABLE 3
Caterpillar Resistance to Deep Freezing for Various Sequences, Rates, and Lengths of Freezing. Captivity Temperature = 40.

MAN ORGETTOR	Jers course	Vinerio 1706- Heri	Последова- тельность оканивания	Раскция после оттакрания	Departs	
22	18 XII	20	(1) 26 MMH. —36° (2) 2 Cyr. —78° (3) MMH. —166°	18% gaurantes, () 18% pearupyot () na as. tok	6-61	
84	28 XII	20	80 mmm	My generates, My pearapyer as es, ton	6,7-30	
53	26 XII	20	30 mms30° 30 mms30° 60 mms10° 130 mms196°	86% ABLYANTER, 10% PERTURYOT HA SA. TOK	7-86	
23	M XII	20	60 MMH. —30° 60 MMH. —78°	04 norbumben 5% pearupyer HS b4, TOX	d-4	
27	27 XII	20	130 MMH. —190° 60 MMH. —50°	0% Dearmenax, or is no 26% pearmpymer ma am. Tex	6/20 10	
*	26 XII	20	€0 мин. —78°	13.3% RENTANTON, 21.8% PARTHPYIST NA SA. TON		

/LEGENDS to Tables 2 and 3 on page 57

The chilling conditions (rate, succession of temperature changes) is of much importance in testing for deep freezing. From the data in Table 3 it is clear that a temperature of -50° results in the death of the caterpillars, that is, the intermediate temperature of -50° for the corn borer is more damaging than -78° and even -196° . The reasons for this have not yet become clear, but it is probable that they stem from the character of water crystallization in the tissues.

The length of residence of the caterpillars at a temperature of -78° essentially does not affect their survival rates for chilling from 30 minutes to five days 100% of live caterpillars were obtained following thawing, and after 25 days of chilling -- 79.2%. Consequently, there is a possibility of preserving in a state of anaviosis whole complex animals, containing from 65-75% water, that has been converted into ice.

We can judge the condition of the cells and organs following deep freezing from dissection of the caterpillars after several hours subsequent to thawing. For caterpillars not showing spontaneous movement following the freezing, motor reactions can be observed in response to electrical stimulus, as well as pulsations of the heart and intestines, respiration and the formation of neutral red granules. Therefore, total immobility following freezing is not a criterion of death; movement can be restored in one to two days following thawing.

Conclusions

The resistance of insects to deep freezing results from many conditions both preceding and during the freezing, as well as following thawing. Chief among these is adaptation to below zero temperatures. This stems from the following: 1) phylogenetic adaptation of the species to low temperatures; 2) seasonal change in cold resistance related first of all with diapause; 3) ontogenetic adaptations — the process of hardening under the influence of ecological factors during the start of hibernation. The adaptation of whole insects to low and ultra-low temperatures is the consequence of those changes occurring in the cells.

Slow chilling increases the survival rate of the insects, but there are dangerous temperature somes which the organism evidently must "traverse" rapidly. The rate of warming is not essentially important for the insects. Of great interest is the study of the conditions under which the repairing of damage arising under the influence of low temperatures takes place.

The resistance of insects to ultra-low temperatures is possible only due to the ability of the tissues and cells with normal water content to survive freezing and the total cessation of metabolism. Vitrification was absent in our experiments. Analysis of the reasons for such stability is reserved for future studies.

The resistance of highly organized animals to deep freezing indicates that diverse cells of various tissues and entire organisms can withstand the environmental conditions which they did not exist under during the process of evolution, and that arising in the cells during the process of adaptation is such a reserve of resistance to extreme incluences as would not be necessary for conditions of earthly existence.

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LEGEND to Table 2/
a) No of experiment; b) Date of experiment; c) Number of caterpillars; d) Captivity temperature in °C; e) before freezing; f) after freezing; g) Reaction following thawing; h) Survival rate and development; i) reacts to elec. current; j) move: about; k) before 28 Dec, °C, since 28 Dec, °2°C; l) Perished in 1 day; m) Lived from 3 to 34 days; n) chrysallised; o) butterflies; p) Lived from 2 to 23 days; q) As above.

LEGEND to Table 3/
a) No of experiment; b) Date of experiment; c) Number of caterpillars; d) Sequence of freezing; e) Reaction following thawing; f) Lifespan in days; g) minutes; h) days; i) move about; j) react to elec. current; k) up to 10.

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